

## CLAIMS

1. Method of screening the operating conditions of a coupling reaction of at least two functional groups, which comprises the following steps:

i) reacting together at least two compounds:

- a first compound of formula  $E_1-X_1-G_1$  in which  $G_1$  represents a first of said at least two functional groups,  $X_1$  represents a covalent bond or a first spacer group, while  $E_1$  represents the residue of a first molecule  $M_1$  for which a first specific antibody  $AC_1$  is available, and

- a second compound of formula  $E_2-X_2-G_2$  in which  $G_2$  represents a second of said at least two functional groups,  $X_2$  represents a covalent bond or a second spacer group, which may be identical to or different from  $X_1$ , while  $E_2$  represents either the residue of a second molecule  $M_2$  that is different from  $M_1$  and for which a second specific antibody  $AC_2$  is available, or a group capable of forming at least one covalent bond with the antibody  $AC_1$  in the presence of a coupling agent;

said at least two compounds being reacted in solution in a solvent and under predetermined operating conditions, at least one of which is a candidate operating condition, in order to obtain a reaction medium and the formation, in this medium, of a compound  $Z$  comprising the chain  $E_1-X_1-G_1-G_2-X_2-E_2$  in which  $X_1$ ,  $X_2$ ,  $E_1$  and  $E_2$  have the same meaning as above, while  $G_1-G_2$

represents the group of atoms resulting from the coupling of said at least two functional groups;

5           ii) determining the concentration of compound Z in the reaction medium at a predetermined reaction time  $t$ , by means of at least one immunoassay using at least the antibody AC<sub>1</sub>; and

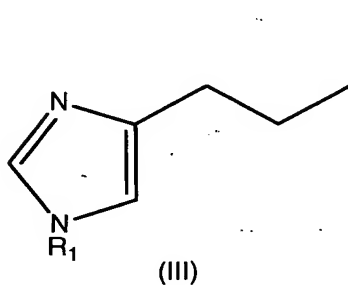
          iii) evaluating the effects of the candidate operating condition(s) on said coupling reaction using the concentration of compound Z thus  
10       determined.

2. Method according to Claim 1, in which the coupling reaction is chosen from the group consisting of esterification reactions, amidation  
15       reactions, aldolization and nitroaldolization reactions, the Heck reaction, the Baylis-Hillman reaction, the Michael reaction, metathesis reactions, the Diels-Alder reaction, the Sonogashira reaction, the Suzuki reaction, the Kumada reaction, the Stille  
20       reaction, the Hiyama reaction, the Liebeskind-Srogl reaction, the Mannich reaction, the Hantzsch reaction, the reaction of Bossio et al., the Ugi reaction, and variants thereof.

25           3. Method according to Claim 1 or Claim 2, in which E<sub>1</sub> or E<sub>2</sub> represents the histamine residue.

          4. Method according to Claim 1 or Claim 2, in which E<sub>1</sub> or E<sub>2</sub> represents the homovanillic acid  
30       residue.

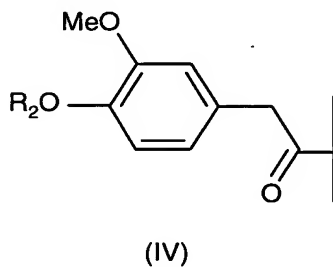
5. Method according to Claim 3, in which  $E_1$  or  $E_2$  corresponds to formula (III) below:



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in which  $R_1$  represents a hydrogen atom or a protective group.

6. Method according to Claim 5, in which  $E_1$  or  $E_2$  corresponds to formula (IV) below:



15 in which  $R_2$  represents a hydrogen atom or a protective group.

7. Method according to Claim 1 or Claim 2, in which  $E_2$  represents a group chosen from amine, carboxylic acid, aldehyde, thiol, phenol, alkenyl and azide groups, and photoactivatable groups.

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8. Method according to Claim 7, in which  $E_2$  represents an amine or thiol group.

9. Method according to any one of Claims 1 to 8, in which said at least one immunoassay for the compound Z is a solid-phase assay.

10. Method according to any one of Claims 1 to 6, in which, since  $E_2$  corresponds to the residue of a molecule  $M_2$ , step ii) comprises the following steps:

a<sub>1</sub>) bringing the reaction medium obtained at reaction time  $t$  into contact with a solid phase on which the first antibody  $AC_1$  is immobilized, so as to obtain the attachment of the compound Z on this solid phase by immunobinding between this antibody and the residue  $E_1$  of this compound;

b<sub>1</sub>) bringing the solid phase into contact with a conjugate comprising the second antibody  $AC_2$  coupled to a label, so as to obtain the attachment of this conjugate to this solid phase by immunobinding between the second antibody  $AC_2$  and the residue  $E_2$  of the compound Z attached to said solid phase;

c<sub>1</sub>) measuring the amount of conjugate attached to the solid phase by means of the label coupled to the antibody  $AC_2$ ; and

d<sub>1</sub>) determining, on a standard range, the concentration of the compound Z in the reaction medium at said time  $t$ , from the amount of conjugate thus measured;

said step ii) also comprising one or more operations consisting in washing the solid phase, between steps a<sub>1</sub>) and b<sub>1</sub>), and between steps b<sub>1</sub>) and c<sub>1</sub>).

5                    11. Method according to any one of Claims 1, 2, 7 or 8, in which, since E<sub>2</sub> corresponds to a group capable of forming at least one covalent bond with the first antibody AC<sub>1</sub>, step ii) comprises the following steps:

10                    a<sub>2</sub>) bringing the reaction medium obtained at reaction time t into contact with a solid phase on which the first antibody AC<sub>1</sub> is immobilized, so as to obtain the attachment of the compound Z to this solid phase by immunobinding between this antibody and the  
15                    residue E<sub>1</sub> of this compound;

                    b<sub>2</sub>) reacting a coupling agent with the first antibody AC<sub>1</sub> immobilized on the solid phase and the group E<sub>2</sub> of the compound Z attached to this solid phase, so as to obtain the formation of one or more  
20                    covalent bonds between this antibody and this group;

                    c<sub>2</sub>) denaturing the immunobond which exists between the first antibody AC<sub>1</sub> immobilized on the solid phase and the residue E<sub>2</sub> of the compound Z attached to this solid phase, so as to release this residue from  
25                    this solid phase;

                    d<sub>2</sub>) bringing the solid phase into contact with a conjugate comprising the first antibody AC<sub>1</sub> coupled to a label, so as to obtain the attachment of this conjugate to this solid phase by immunobinding  
30                    between said antibody and the residue E<sub>1</sub> of the compound E<sub>1</sub>-X-G<sub>1</sub>-G<sub>2</sub>-Y-E<sub>2</sub> thus released;

e<sub>2</sub>) measuring the amount of conjugate attached to the solid phase by means of the label coupled to the antibody AC<sub>1</sub>; and

5 f<sub>2</sub>) determining, on a standard range, the concentration of compound Z in the reaction medium at said time t, from the amount of conjugate thus measured;

10 said step ii) also comprising one or more operations consisting in washing the solid phase, between steps a<sub>2</sub>) and b<sub>2</sub>), b<sub>2</sub>) and c<sub>2</sub>), c<sub>2</sub>) and d<sub>2</sub>), and between steps d<sub>2</sub>) and e<sub>2</sub>).

12. Method according to any one of the preceding claims, in which the first antibody AC<sub>1</sub> is a  
15 monoclonal antibody.

13. Method according to any one of Claims 1 to 6 or 10, in which the second antibody AC<sub>2</sub> is a  
20 monoclonal antibody.

14. Method according to any one of the preceding claims, in which the solid phase is the wall of a well of a microtitration plate onto which the first antibody AC<sub>1</sub> is adsorbed.

25 15. Method according to Claim 10 or Claim 11, in which the label is an enzyme, preferably acetylcholine esterase.

30 16. Method according to any one of the preceding claims, which comprises an operation

consisting of dilution of the reaction medium between steps i) and ii).

17. Method according to any one of the preceding claims, in which the yield of the coupling reaction is determined from the concentration of compound Z in the reaction medium.

18. Method according to any one of the preceding claims, in which the coupling reaction consists in coupling 2, 3 or 4 functional groups.

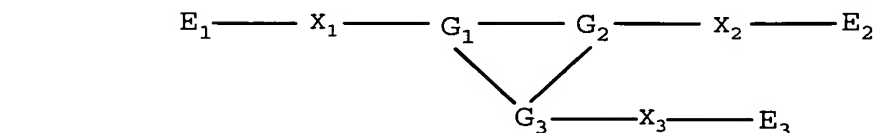
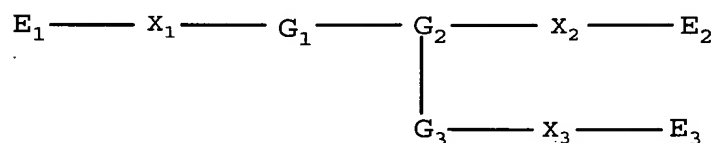
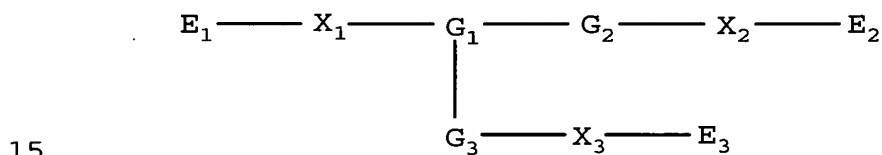
19. Method according to Claim 18, in which the coupling reaction consists in coupling two functional groups  $G_1$  and  $G_2$ , and in which:

- in step i), the compounds of formulae  $E_1-X_1-G_1$  and  $E_2-X_2-G_2$  are reacted together so as to obtain the formation, in the reaction medium, of a compound Z which corresponds to the formula  $E_1-X_1-G_1-G_2-X_2-E_2$  in which  $X_1$ ,  $X_2$ ,  $E_1$  and  $E_2$  have the same meaning as above and  $G_1-G_2$  represents the group of atoms resulting from the coupling between said functional groups  $G_1$  and  $G_2$ ; while

- in step ii), the concentration of compound Z in the reaction medium is determined by means of a single immunoassay.

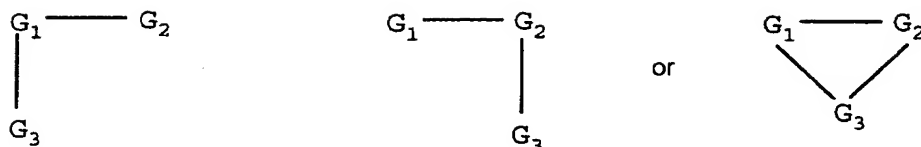
20. Method according to Claim 18, in which the coupling reaction consists in coupling three functional groups  $G_1$ ,  $G_2$  and  $G_3$ , and in which:

- in step i), the compounds of formulae  $E_1-X_1-G_1$  and  $E_2-X_2-G_2$  are reacted with a third compound of formula  $E_3-X_3-G_3$  in which  $X_3$  represents a covalent bond or a third spacer group, which may be identical to or different from  $X_1$  and/or  $X_2$ , while  $E_3$  represents either the residue of a third molecule  $M_3$  which is different from  $M_1$  and from  $M_2$  and for which a third specific antibody  $AC_3$  is available, or a group capable of forming a covalent bond with the antibody  $AC_1$  in the presence of a coupling agent on the condition, however, that  $E_2$  does not already represent such a group, so as to obtain the formation, in the reaction medium, of a compound Z corresponding to one of the formulae below:



in which  $X_1$ ,  $X_2$ ,  $X_3$ ,  $E_1$ ,  $E_2$  and  $E_3$  have the same meaning as above, and





represents the group of atoms resulting from the coupling of said functional groups  $G_1$ ,  $G_2$  and  $G_3$ ; while

5                    - in step ii), the concentration of compound Z in the reaction medium is determined by means of two different immunoassays.

21. Method according to Claim 18, in which

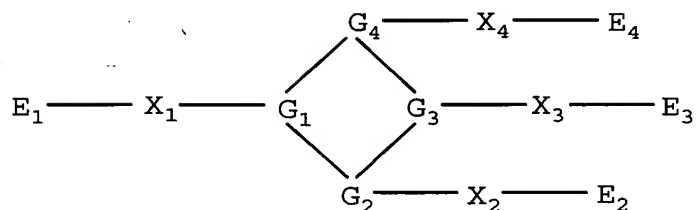
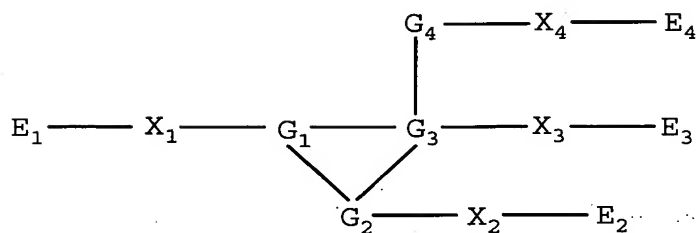
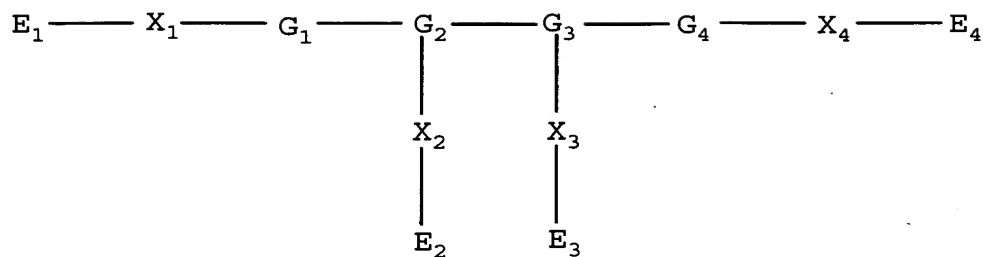
10    the coupling reaction consists in coupling four functional groups  $G_1$ ,  $G_2$ ,  $G_3$  and  $G_4$ , and in which:

                  - in step i), the compounds of formula  $E_1-X_1-G_1$  and  $E_2-X_2-G_2$  are reacted with a third compound of formula  $E_3-X_3-G_3$  as defined above and a fourth

15    compound of formula  $E_4-X_4-G_4$  in which  $X_4$  represents a covalent bond or a fourth spacer group, which may be identical to or different from  $X_1$ ,  $X_2$  and/or  $X_3$ , while  $E_4$  represents either the residue of a third molecule  $M_4$  which is different from  $M_1$ , from  $M_2$  and from  $M_3$  and for

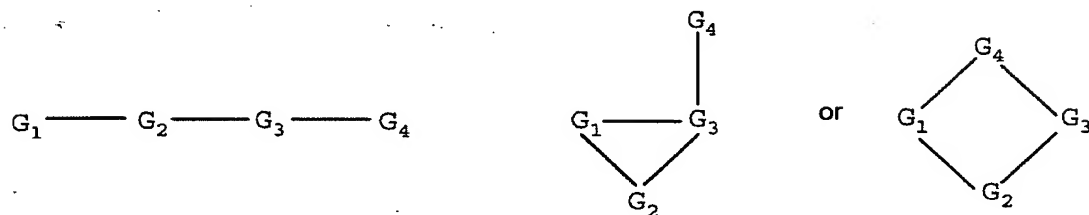
20    which a fourth specific antibody  $AC_4$  is available, or a group capable of forming a covalent bond with the antibody  $AC_1$  in the presence of a coupling agent, on the condition, however, that  $E_2$  and  $E_3$  do not already represent such a group, so as to obtain the formation,

25    in the reaction medium, of a compound Z corresponding to one of the formulae below:



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in which  $X_1$ ,  $X_2$ ,  $X_3$ ,  $X_4$ ,  $E_1$ ,  $E_2$ ,  $E_3$  and  $E_4$  have the same meaning as above, and



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represents the group of atoms resulting from the coupling of said functional groups  $G_1$ ,  $G_2$ ,  $G_3$  and  $G_4$ ; while

- in step ii), the concentration of compound Z in the reaction medium is determined by means of three different immunoassays.

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22. Method according to any one of the preceding claims, in which the candidate operating condition(s) is(are) chosen from the group consisting of solvents, catalysts, temperature levels, pressure levels, the use of ultrasound, concentrations, stoichiometric ratios, reaction times and combinations thereof.

23. Method according to any one of the preceding claims, in which the candidate operating condition(s) is(are) catalysts.

24. Kit for carrying out a method of screening the operating conditions of a coupling reaction of at least two functional groups, which comprises suitable amounts:

- of at least two compounds intended to react together:

• a first compound of formula  $E_1-X_1-G_1$  in which  $G_1$  represents a first of said at least two functional groups,  $X_1$  represents a covalent bond or a first spacer group and  $E_1$  represents the residue of a first molecule  $M_1$ ; and

• a second compound of formula  $E_2-X_2-G_2$  in which  $G_2$  represents a second of said at least two functional groups,  $X_2$  represents a covalent bond or a second spacer group, which may be identical to or different from  $X_1$ , and  $E_2$  represents the residue of a second molecule  $M_2$  which is different from  $M_1$ ;

- of at least two antibodies:

- a first antibody  $AC_1$  specific for the first molecule  $M_1$ , this antibody being optionally attached to a plurality of solid phases; and
  - a second antibody  $AC_2$  specific for the second molecule  $M_2$ , this antibody being coupled to a label;
    - of a compound Z comprising the chain  $E_1-X_1-G_1-G_2-X_2-E_2$  in which  $X_1$ ,  $X_2$ ,  $E_1$  and  $E_2$  have the same meaning as above, while  $G_1-G_2$  represents the group of atoms resulting from the coupling of said at least two functional groups; and, optionally:
      - of a reagent for visualizing the label, for example a substrate if the label is an enzyme; and
      - of suitably chosen buffers.
25. Kit for carrying out a method of screening the operating conditions of a coupling reaction of at least two functional groups, which comprises suitable amounts:
- of at least two compounds intended to react together:
    - a first compound of formula  $E_1-X_1-G_1$  in which  $G_1$  represents a first of said at least two functional groups,  $X_1$  represents a covalent bond or a first spacer group and  $E_1$  represents the residue of a first molecule  $M_1$ ; and
    - a second compound of formula  $E_2-X_2-G_2$  in which  $G_2$  represents a second of said at least two functional groups,  $X_2$  represents a covalent bond or a second spacer group, that may be identical to or different from  $X_1$ , and  $E_2$  represents a group capable of forming one or more covalent bonds with an antibody specific

for the molecule  $M_1$  in the presence of a coupling agent;

- of at least one antibody, this antibody being said antibody specific for the molecule  $M_1$ ;

5       - of a conjugate comprising said antibody specific for the molecule  $M_1$  coupled to a label;

      - of a compound Z comprising the chain  $E_1-X_1-G_1-G_2-X_2-E_2$  in which  $X_1$ ,  $X_2$ ,  $E_1$  and  $E_2$  have the same meaning as above, while  $G_1-G_2$  represents the group of  
10       atoms resulting from the coupling of said at least two functional groups; and, optionally:

      - of a reagent for visualizing the label,

      - of a coupling agent,

      - of a reagent capable of denaturing an  
15       immunobond, and

      - of suitably chosen buffers.

26. Use of a screening method according to any one of Claims 1 to 23 or of a kit according to  
20       Claim 24 or Claim 25, for the screening, in particular the "high-throughput" screening, of catalysts that are useful in a coupling reaction between two functional groups.